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Role of proteinase-activated receptor-1 gene polymorphisms in susceptibility to chronic obstructive pulmonary disease

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ABSTRACT. We conducted a case-control study to investigate the association between *PAR1* gene polymorphisms and the development of chronic obstructive pulmonary disease (COPD). A total of 270 patients with COPD and 270 control subjects were consecutively recruited between March 2012 and March 2014. A polymerase chain reaction restriction fragment length polymorphism assay was used to assess the polymorphisms PAR1 IVS-14 A/T rs168753 and -506 I/D rs11267092. The frequency of the AA genotype in PAR1 IVS-14 A/T rs168753 was significantly higher than in the controls (χ^2 = 7.23, P = 0.03). By logistic regression analysis, we found that the AA genotype of PAR1 IVS-14 A/T rs168753 was associated with increased risk of COPD compared with the GG genotype. The adjusted OR (95%CI) was 2.00 (1.15-3.50) for the AA genotype. In conclusion, we found that the PAR1 IVS-14 A/T rs168753 polymorphism was associated with the development of COPD.

Key words: Proteinase-activated receptor-1; Polymorphism; Chronic obstructive pulmonary disease

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) causes airflow obstruction by inflaming the airways, as well as other systemic effects and comorbidities. It is estimated that COPD will become the third leading cause of death worldwide by 2020, and it could place a great burden on patients' families and on healthcare systems (Murray and Lopez, 1997). The pathogenesis of COPD is complex and involves many environmental factors, but it is reported that inhalation of tobacco smoke is the most important exogenous risk factor in the development of COPD (Decramer et al., 2012). However, less than 20% of long-term smokers develop symptomatic airflow obstruction during their lifetime (Fletcher and Peto, 1977; Faner et al., 2014). Therefore, it is hypothesized that genetic factors may contribute to an individual's susceptibility to COPD. There is increasing evidence that genetic factors, such as *VDBP*, *MDR1*, *COX2*, and *MMP12*, may increase susceptibility to COPD in addition to long-term smoking (Toru et al., 2014; Yu et al., 2014; Horita et al., 2015).

Protease-activated receptors (PARs) are members of the G protein-coupled receptor superfamily. There are four different receptors: PAR-1, PAR-2, PAR-3, and PAR-4. Of these, PAR-1 contributes to many pathophysiological processes such as growth, development, mitogenesis, and inflammation (Déry et al., 1998). The inflammatory effects mediated by PAR-1 include vasodilatation, vasoconstriction, increased vascular permeability, cellular adhesion, chemotaxis, and activation of a T-helper type 1 cytokine profile in the gastrointestinal tract (Déry et al., 1998; Cirino and Vergnolle, 2006). Single-nucleotide polymorphisms (SNPs) in the *PAR1* gene may alter the expression of PAR-1, and thereby influence the development of COPD. We therefore conducted a case-control study to investigate the association between *PAR1* gene polymorphisms and the development of COPD.

MATERIAL AND METHODS

Study population

A total of 270 patients with COPD and 270 control subjects were consecutively recruited from the Tai'an Central Hospital of Shandong Province between March 2012 and March 2014 for this case-control study. Patients with COPD were diagnosed according to the criteria specified by the World Health Organization's Global Initiative for Chronic Obstructive Lung Disease (Rabe et al., 2007). Patients who had other respiratory diseases, such as lung cancer, pulmonary tuberculosis, cystic fibrosis, or bronchial asthma, were excluded from our study.

Smoking status was divided into smokers and non-smokers. Smokers were defined as those who smoked more than one cigarette/pipe per day for at least half a year. Alcohol consumption status was divided into drinkers and non-drinkers. Alcohol drinkers were defined as those who consumed 50 g alcohol (200 mL beer, 100 mL wine, or 50 mL white spirit) per week for at least half a year. Family history referred to first- or second-degree relatives.

The demographic and clinical data were collected using a questionnaire that we designed. It comprised questions about age, gender, cigarette smoking, alcohol consumption, and family history of COPD. Written informed consent was obtained from each subject before they entered the study group. The study was approved by the Institute Research Ethics Committee of Tai'an Central Hospital of Shandong Province prior to commencement.

DNA extraction and genotyping

Before the patients began their treatment, 2 mL peripheral blood was collected with ethylenediaminetetraacetic acid (EDTA)-anticoagulant tubes. Genomic DNA was extracted from the blood using a QIAamp DNA MAX Kit (Qiagen, Hilden, Germany) according to the manufacturer instructions. A polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was applied to assess the polymorphisms PAR1 IVS-14 A/T rs168753 and -506 I/D rs11267092. PCR reactions were carried out with an initial denaturation step of 8 min at 94°C, followed by 30 cycles at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. The resulting DNA fragments were electrophoresed on 3.5% agarose gel and visualized under UV light after ethidium staining. Moreover, about 5% of the samples were randomly selected to retest, and the results were 100% concordant.

Statistical analysis

Statistically significant differences between cases and controls for demographic characteristics were assessed by Student's *t*-test and the χ^2 test. Hardy-Weinberg equilibrium (HWE) was assessed for PAR1 IVS-14 A/T rs168753 and -506 I/D rs11267092 gene polymorphisms using a standard χ^2 test or Fisher's exact test. Logistic regression analysis was used to estimate the odds ratios (ORs) and the corresponding 95% confidence intervals (95%CIs) for associations between the genotypes and the risk of COPD. All P values were two-sided, and a P value < 0.05 was considered statistically significant. All analyses were performed using the SPSS 16.0 software (version 16.0, SPSS Inc., USA).

RESULTS

He characteristics of the COPD patients and controls are shown in Table 1. The mean ages were 67.80 ± 11.50 years for the COPD patients and 67.20 ± 11.70 years for the control subjects. No statistically significant differences were found between the COPD patients and controls regarding sex, age, and alcohol consumption ($P > 0.05$). We found that the COPD patients were more likely to be cigarette smokers and have a family history of COPD ($P < 0.05$).

The distribution of PAR1 IVS-14 A/T rs168753 and -506 I/D rs11267092 polymorphisms in the COPD patients and controls is shown in Table 2. The frequencies of the GG, GA, and AA genotypes in PAR1 IVS-14 A/T rs168753 were 120 (44.44%), 102 (37.78%), and 48 (17.78%), respectively, in the patients, and 145 (53.70%), 96 (35.56%), and 29 (10.74%), respectively, in the controls (Table 2). The frequencies of the del/del, ins/del, and ins/ins genotypes in PAR1-506 I/D rs11267092 were 239 (88.52%), 31 (11.48), 0 (0.00%), respectively, in the patients, and 247 (91.48%), 23 (8.52%), and 0 (0.00), respectively, in the controls. The frequency of AA genotypes in PAR1 IVS-14 A/T rs168753 was significantly higher than in the controls ($\chi^2 = 7.23$, $P = 0.03$). The genotype distributions of PAR1 IVS-14 A/T rs168753 and PAR1-506 I/D rs11267092 were in HWE in the controls.

By logistic regression analysis, we found that the AA genotype of PAR1 IVS-14 A/T rs168753 was associated with increased risk of COPD compared with the GG genotype (Table 3). The adjusted OR (95%CI) was 2.00 (1.15-3.50) for the AA genotype. However, no significant positive association was observed between the PAR1-506 I/D rs11267092 polymorphism and risk of COPD.

Table 1. Characteristics of chronic obstructive pulmonary disease (COPD) patients and control subjects.

Variables	COPD patients	%	Control subjects	%	χ^2 test	P value
Age						
Mean age, years	67.80 ± 11.50		67.20 ± 11.70		0.60	0.27
<65	125	46.30	124	45.93		
≥65	145	53.70	146	54.07	0.008	0.93
Sex						
Female	85	31.48	85	31.48		
Male	185	68.52	185	68.52	0.00	1.00
Cigarette smoking						
Non-smokers	141	52.22	166	61.48		
Smokers	129	47.78	104	38.52	4.72	0.03
Alcohol consumption						
Non-drinkers	144	53.33	150	55.56		
Drinkers	126	46.67	120	44.44	0.27	0.60
Family history of COPD						
No	260	96.30	268	99.26		
Yes	10	3.70	2	0.74	5.45	0.02

Table 2. Genotype frequencies of PAR1 IVS-14 A/T rs168753 and -506 I/D rs11267092 in chronic obstructive pulmonary disease (COPD) patients and control subjects.

Genotypes	Patients	%	Controls	%	χ^2	P value	HWE
PAR1 IVS-14 A/T rs168753							
GG	120	44.44	145	53.70			
GA	102	37.78	96	35.56			
AA	48	17.78	29	10.74	7.23	0.03	0.07
PAR1-506 I/D rs11267092							
del/del	239	88.52	247	91.48			
ins/del	31	11.48	23	8.52			
ins/ins	0	0.00	0	0.00	1.32	0.32	0.46

HWE = Hardy-Weinberg equilibrium.

Table 3. Association between PAR1 IVS-14 A/T rs168753 and -506 I/D rs11267092 gene polymorphisms and risk of chronic obstructive pulmonary disease (COPD).

Polymorphism	Patients	%	Controls	%	OR (95%CI)[a]	P value
PAR1 IVS-14 A/T rs168753						
GG	120	44.44	145	53.70	Ref.	
GA	102	37.78	96	35.56	1.28 (0.87-1.89)	0.18
AA	48	17.78	29	10.74	2.00 (1.15-3.50)	0.01
PAR1-506 I/D rs11267092						
TT	239	88.52	247	91.48	Ref.	
TC	31	11.48	23	8.52	1.39 (0.76-2.58)	0.25
CC	0	0.00	0	0.00	NA	NA

[a]Adjusted for sex, age, cigarette smoking, and family history of COPD.

DISCUSSION

Genetic susceptibility to disease is well known, and the investigation of the involvement of gene polymorphisms in various diseases has received increasing attention. PAR-1 is involved in mediating the interplay between coagulation and inflammation, and the gene that encodes it, *PAR1*, has attracted increasing interest as a candidate gene in the pathogenesis of COPD (Chambers and Scotton, 2012; Mercer and Chambers, 2013). Previous studies have reported that *PAR1*

gene polymorphisms are associated with various diseases, such as venous thromboembolism, coronary heart disease, liver fibrosis, and myocardial infarction (Park et al., 2000; Smith et al., 2005; Martinelli et al., 2008; Gigante et al., 2009). In our study, we found that the AA genotype of PAR1 IVS-14 A/T rs168753 was associated with the development of COPD.

It has been reported that frequent COPD exacerbation may be correlated with increased inflammatory responses, and IL-6 and fibrinogen levels play an important role in increasing exacerbation (Wedzicha et al., 2000). Therefore, there is a hypothesis that higher levels of *PAR1* gene expression mediate the inflammatory response and cause resistance to COPD exacerbation. Previously, studies have reported an association between *PAR1* gene polymorphisms and the development of many diseases, such as renal cell carcinoma and premature myocardial infarction (Motovska et al., 2010; de Martino et al., 2013). de Martino et al. (2013) reported that the AA genotype of the PAR1 IVS-14 A/T rs168753 polymorphism is associated with an elevated risk of metastasis in renal cell carcinoma. Motovska et al. (2010) conducted a study to evaluate the role of hemostatic and platelet receptor gene polymorphisms in myocardial infarction, and did not find a significant association between PAR1 IVS-14 A/T rs168753 and the risk of premature onset of myocardial infarction.

To the best of our knowledge, this is the first time the association between PAR1 IVS-14 A/T rs168753 and the risk of COPD has been evaluated. One previous study has been conducted on the association between the PAR1 rs2227744G>A polymorphism and the risk of COPD (Platé et al., 2014). In this study, the researchers found that the PAR1 rs2227744G>A polymorphism plays an important role in the development of COPD, and protects against COPD exacerbations (Platé et al., 2014). Therefore, further studies to confirm the association between PAR1 IVS-14 A/T rs168753 and the risk of COPD would be very useful.

Two limitations should be considered in our study. First, other genetic polymorphisms besides those in the *PAR1* gene may influence the development of COPD. Second, the present sample size was relatively small, although the number of study participants met the requirement for analysis. Therefore, larger sample studies are required in the future.

In conclusion, we found that the PAR1 IVS-14 A/T rs168753 polymorphism was associated with the development of COPD. Further well-designed studies with large sample sizes are needed to confirm our results.

Conflicts of interest

The authors declare no conflict of interest.

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